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Synthesis and antimicrobial studies of novel 1-benzhydryl-piperazine sulfonamide and carboxamide derivatives

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Abstract

A series of novel substituted 1-benzhydryl-piperazine sulfonamide **8(a–f)** and benzamides **9(a–h)** were synthesized and their antimicrobial activities evaluated *in vitro* by paper disc diffusion and micro dilution method against standard strains of Gram-positive (*Staphylococcus aureus* ATCC 25953, *Staphylococcus epidermis* 25212, *Bacillus cereus* 11778, *Bacillus substilis* 6051) and Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, *Proteus vulgaris* ATCC 2853 and *Salmonella typhi* ATCC 9484) bacteria. Among the synthesized new compounds **8d, 8e, 9c, 9e**, **9f** and **9 h** showed potent antimicrobial activities compared to the standard drug streptomycin.

Keywords: *1-benzhydryl-piperazine derivatives, sulfonyl chlorides, acid chlorides, antimicrobials*

Introduction

Diseases caused by microbial infection are a serious menace to the health of human beings and often have connection to some the other diseases (opportunistic), whenever the body system gets debilitated. In order to combat these diseases a number of drugs are available in clinical practice, ranging from natural product antibacterials to tailor-made antibacterial drugs. Developing antimicrobial drugs and maintaining their potency, inopposition to resistance by different classes of microorganisms as well as a broad spectrum of antibacterial activity are some of the major concern of research in this area. The scaffold piperazine and its analogues are an important pharmacophore that can be found in biologically active compounds across a number of different therapeutic areas [1]. These include anticancer [2–3], antimicrobial [4], anti-malarial, antipsychotic agents [5], HIV protease inhibitors [6–8], antidepressants [9] and antitumor drugs against colon, prostate, breast, lung and leukemia cancers [10]. In 1935 Domargk showed the therapeutic value of the sulfonamides. These substances are not specific to group of organisms, but are effective against a large variety of pathogenic organisms. Sulfonamides are among the most widely used antibacterial agents [11] and can produce a variety of outward effects due partly to allergy, direct toxicity, allergic nephritis and anaemia [12]. Piperazine sulfonamides exhibit diverse therapeutic activities such as antibacterial activity, matrix metalloprotein-3 inhibition and carbonic anhydrase inhibition [13].

Compounds containing an amide bond with fluorine atom substitution can alter the chemical properties, disposition and biological activities of drugs [14]. Amides are currently used antidepressants, anti-inflammatory agents, antimalarial agents, antipsychotic agents, antiviral agents, steroids and general anesthetics [15]. Amide functional groups are also found in many antibacterial agents e.g. benzimidazole carboxamides, peptide, penicillin, cephalosporins and thiozolidinones [16]. Recently we have

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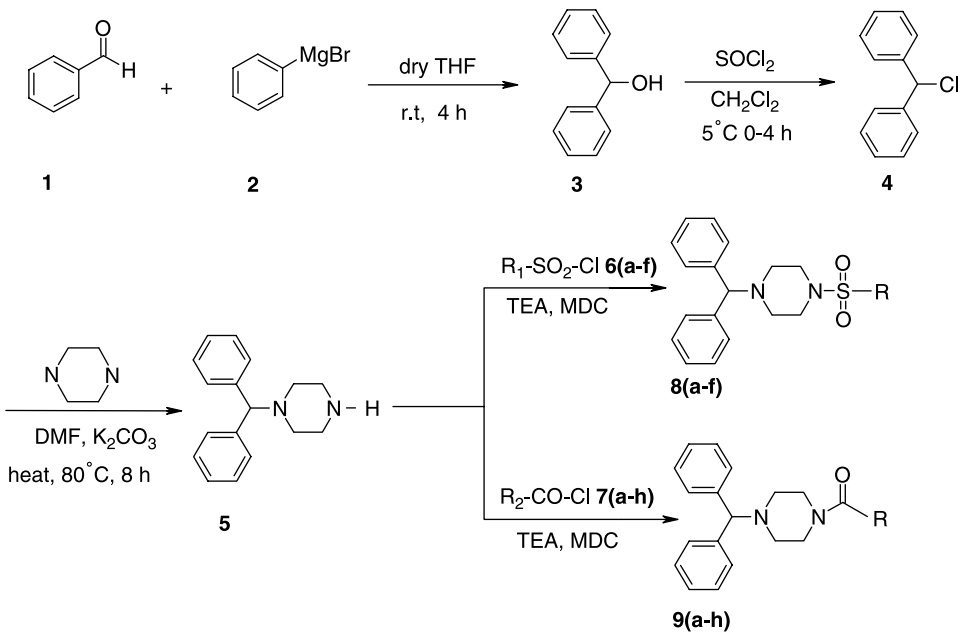
reported the synthesis and antimicrobial activity of bioactive heterocyclic sulfonamides and benzamides [17–19]. In continuation of our research on the synthesis of bioactive heterocycles and their biological evaluation, we describe here the synthesis of 1-benzhydryl-piperazine derivatives **8(a–f)** and **9(a–h)** along with their *invitro* antimicrobial activity by paper disc diffusion and micro dilution method.

Materials and methods

Chemistry

Melting points were determined using Veego model VMP-III melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Jasco FTIR-4100 series. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker AM-400, and chemical shifts are expressed in parts per million (ppm, for δ) relative to tetra methyl silane as an internal standard and DMSO-d6 as solvent. Spin multiplets are given as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Elemental (CHNS) analysis was obtained on Vario EL III Elementar. Silica gel column chromatography was performed using Merck 7734 silica gel (60-120 mesh) and Merck made TLC plates. All of the reagents and chemicals were purchased from Sigma Aldrich Chemicals Pvt Ltd.

1-benzhydryl-piperazine derivatives **8(a–f)** and **9(a–h)** were prepared by the method summarized in **Scheme 1**. The reaction between benzaldehyde **(1)** with phenylmagnesium bromide **(2)** under nitrogen atmosphere gave benzhydrol **(3)**. Compound **3** was subsequently treated with thionyl chloride to give the corresponding benzhydryl chloride **(4)** which was directly treated with piperazine and anhydrous potassium carbonate using dimethyl formamide as a solvent at 80°C gave target key intermediate 1-benzhydryl-piperazine **(5).** The nucleophilic substitution reaction of 1-benzhydryl-piperazine **(5)** with different substituted aromatic sulfonyl chlorides



Where (R_1-SO_2-Cl) are

(6a) = 2-nitro-benzenesulfonyl chloride

(6b) = 3-nitro-benzenesulfonyl chloride

(6c) = 4-nitro-benzenesulfonyl chloride

(6d) = 2, 5-dichloro-benzenesulfonyl chloride

(6e) = 4-chloro-benzenesulfonyl chloride

(6f) = 4-tertiarybutyl-benzenesulfonyl chloride

Where (R_2-CO-Cl) are

(7a) = 4-tertiarybutyl-benzoyl chloride

(7b) = 4-chloro-benzoyl chloride

(7c) = 2, 4-dichloro-benzoyl chloride

(7d) = 3-bromo-benzoyl chloride

(7e) = 2-fluoro-benzoyl chloride

(7f) = 2,6-difluoro-benzoyl chloride

(7g) = 3-methoxy-benzoyl chloride

(7h) = 3,5-dinitro-benzoyl chloride

Scheme 1. Synthesis of titled compounds.

(R_1—SO_2—Cl) and acid chlorides (R_2—CO—Cl) were carried out in presence of triethylamine and dichloromethane as solvent with a good yield ranging from 65–90%. The absence of N—H proton peak in synthesized derivatives **8(a–f)** and **9(a–h)** in proton NMR and IR spectra confirms our products. It is also confirmed by IR data, for sulfonamide series **8(a–f)** which showed asymmetric stretching frequency of O=S=O in the range 1350—1370 cm^{-1} and symmetric stretching frequency at 1270–1290 cm^{-1} and similarly for carboxamide series **9(a–h),** IR data showed stretching frequency of —C=O at 1630–1670 cm^{-1}.

General procedure for synthesis of 1-benzhydryl-piperazine derivatives 8(a–f) and 9(a–h). A solution of 1-benzhydryl-piperazine **5** (1.0 eq) in dry dichloromethane was cooled to 0–5°C in an ice bath. Triethylamine (3 eq) was added to the cold reaction mixture and stirred for 10 min then appropriate sulfonyl chlorides (1.0 eq) or acid chloride (1.0 eq) were added and the reaction mixture was allowed to stirr at room temperature for 5–6 h. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and the residue was taken in water and extracted with ethyl acetate. The organic layer was washed with 10% ammonium chloride solution and finally water, then dried with anhydrous sodium sulphate. The solvent was evaporated to give the crude product which was purified by column chromatography over silica gel (60–120mesh) using hexane: ethyl acetate (8:2) as an eluent.

1-benzhydryl-4-(2-nitro-benzensulfonyl)-piperazine (8a). The general synthetic method described above afforded **8a**, and the product obtained was white crystalline solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 2-nitro-benzenesulfonyl chloride **6a** (0.439 g, 1.98 mmol). Yield (%): 78, M.P (°C): 160–162. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.92–7.99 (m, 3H, Ar-H), 7.80 (t, 1H, J = 1.16 Hz, Ar-H), 7.38 (d, 4H, J = 7.28 Hz, Ar-H), 7.27 (t, 4H, J = 7.36 Hz, Ar-H), 7.15 (t, 2H, J = 7.24 Hz, Ar-H), 4.35 (s, 1H, —CH), 3.2 (br s, 4H, —CH_2—), 2.35 (br s, 4H, —CH_2—). IR (KBr, cm^{-1}): 3106, 2924, 1529, 1350, 1285. Anal. calcd. for $C_{23}H_{23}N_3O_4S$ (in %): C-63.14, H-5.30, N-9.6, S-7.33. Found C-63.10, H-5.25, N-9.3, S-7.29%.

1-benzhydryl-4-(3-nitro-benzensulfonyl)-piperazine (8b). The general synthetic method described above afforded **8b**, and the product obtained was off white crystalline solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 3-nitro-benzenesulfonyl chloride **6b** (0.439 g, 1.98 mmol). Yield (%): 70, M.P (°C): 173–175. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 8.0 (d, 1H, J = 2.0 Hz, Ar-H), 7.65–7.6 (m, 3H, Ar-H), 7.4 (d, 4H, J = 7.30, Ar-H), 7.25 (t, 4H, J = 7.34 Hz, Ar-H), 7.15 (t, 2H, J = 7.23 Hz, Ar-H), 4.32 (s, 1H, —CH), 3.30 (br s, 4H, —CH_2—), 2.4 (br s, 4H, —CH_2—). IR (KBr, cm^{-1}): 3081, 2965, 1530, 1358, 1282. Anal. calcd. for $C_{23}H_{23}N_3O_4S$ (in %): C-63.14, H-5.30, N-9.6, S-7.33. Found C-63.11, H-5.25, N-9.3, S-7.28%.

1-benzhydryl-4-(4-nitro-benzensulfonyl)-piperazine (8c). The general synthetic method described above afforded **8c**, and the product obtained was white crystalline solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 4-nitro-benzenesulfonyl chloride **6c** (0.439 g, 1.98 mmol). Yield (%): 75, M.P (°C): 217–219. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.7–7.85 (m, 4H, Ar-H), 7.4 (d, 4H, J = 7.25 Hz, Ar-H), 7.28 (t, 4H, J = 7.35 Hz, Ar-H), 7.15 (t, 2H, J = 7.23 Hz, Ar-H), 4.32 (s, 1H, —CH—), 3.3 (br s, 4H, —CH_2—), 2.41 (br s, 4H, —CH_2—). IR (KBr, cm^{-1}): 3073, 2961, 1536, 1358, 1283. Anal. calcd.for $C_{23}H_{23}N_3O_4S$ (in %): C-63.14, H-5.30, N-9.6, S-7.33. Found C-63.10, H-5.24, N-9.2, S-7.29%.

1-benzhydryl-4-(2,5-dichloro-benzensulfonyl)-piperazine (8d). The general synthetic method described above afforded **8d**, and the product obtained was white amorphous solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 2,5-dichloro-benzenesulfonyl chloride **6d** (0.486 g, 1.98 mmol).Yield (%): 80, M.P (°C): 136–138. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.9 (d, 1H, J = 2.28 Hz, Ar-H), 7.75-7.8 (m, 2H, Ar-H), 7.4 (d, 4H, J = 7.24 Hz, Ar-H), 7.25 (t, 4H, J = 7.60 Hz, Ar-H), 7.16 (t, 2H, J = 7.2 Hz, Ar-H), 4.33 (s, 1H, —CH—), 3.25 (br s, 4H, —CH_2—), 2.32 (br s, 4H, —CH_2—). IR (KBr, cm^{-1}): 2978, 2852, 1350, 1280, 752. Anal. calcd. for $C_{23}H_{22}Cl_2N_2O_2S$ (in %): C-59.87, H-4.81, N-6.07, S-6.95. Found C-59.82, H-4.78, N-6.04, S-6.90%.

1-benzhydryl-4-(4-chloro-benzensulfonyl)-piperazine (8e). The general synthetic method described above afforded **8e**, and the product obtained was white amorphous solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 4-chloro-benzenesulfonyl chloride **6e** (0.418 g, 1.98 mmol). Yield (%): 76, M.P (°C): 153–155. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.7–7.8 (m, 4H, Ar-H), 7.4 (d, 4H, J = 7.27 Hz, Ar-H), 7.25 (t, 4H, J = 7.36 Hz, Ar-H), 7.16 (t, 2H, J = 7.24 Hz, Ar-H), 4.32 (s, 1H, —CH), 3.31 (br s, 4H, —CH_2—), 2.40 (br s, 4H, —CH_2—). IR (KBr, cm^{-1}): 2961, 2889, 1350, 1279, 707. Anal. calcd.for $C_{23}H_{23}ClN_2O_2S$ (in %): C-59.87, H-4.81, N-6.07, S-6.95. Found C-59.82, H-4.78, N-6.04, S-6.90%.

1-benzhydryl-4-(4-tert-butyl-benzensulfonyl)-piperazine (8f). The general synthetic method described above afforded **8f**, and the product obtained was white amorphous solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 4-tert-butyl-benzenesulfonyl chloride **6f** (0.461 g, 1.98 mmol). Yield (%): 90, M.P (°C): 198–

200. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.63–7.7 (m, 4H, Ar-H), 7.35 (d, 4H, J = 7.24 Hz, Ar-H), 7.26 (t, 4H, J = 7.28 Hz, Ar-H), 7.16 (t, 2H, J = 7.18 Hz, Ar-H), 4.3 (s, 1H, —CH), 2.9 (br s, 4H, —CH_2—), 2.35 (br s, 4H, —CH_2—), 1.3 (s, 9H, (—CH_3)$_3$). IR (KBr, cm^{-1}): 3028, 2852, 1346, 1279, 1399. Anal. calcd. for $C_{23}H_{32}N_2O_2S$ (in %): C-72.29, H-7.19, N-6.24, S-7.15. Found C-72.25, H-7.16, N-6.19, S-7.10%.

(4-benzhydryl-piperazin-1-yl)-(4-tert-butyl-phenyl)-methanone (9a). The general synthetic method described above afforded **9a**, and the product obtained was off white crystalline solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 4-tert-butyl-benzoyl chloride **7a** (0.38 g, 1.98 mmol). Yield (%): 68, M.P(°C):188–190. ^{1}H NMR(DMSO-d6, 400 MHz) δ: 7.63–7.8 (m, 4H, Ar-H), 7.36 (d, 4H, J = 7.24 Hz, Ar-H), 7.25 (t, 4H, J = 7.4 Hz, Ar-H), 7.15 (t, 2H, J = 7.06 Hz, Ar-H), 4.32 (s, 1H, —CH—), 3.67 (br s, 2H, —CH_2), 3.24 (br s, 2H, —CH_2-), 2.34 (br s, 2H, —CH_2—), 2.23 (br s, 2H, —CH_2—), 1.33 (s, 9H, (—CH_3)$_3$). IR (KBr, cm^{-1}): 2961, 2857, 1634, 1394. Anal. calcd. for $C_{28}H_{32}N_2O$ (in %): C-81.51, H-7.82, N-6.79. Found C-81.47, H-7.78, N-6.74%.

(4-benzhydryl-piperazin-1-yl)-(4-chloro-phenyl)-methanone (9b). The general synthetic method described above afforded **9b**, and the product obtained was oily from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 4-chloro benzoyl chloride **7b** (0.34 g, 1.98 mmol). Yield(%):70. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.7–7.8 (m, 4H, Ar-H), 7.4 (d, 4H, J = 7.27 Hz, Ar-H), 7.26 (t, 4H, J = 7.34 Hz, Ar-H), 7.16 (t, 2H, J = 7.26 Hz, Ar-H), 4.3 (s, 1H, —CH), 3.67 (br s, 2H, —CH_2), 3.24 (br s, 2H, —CH_2), 2.34 (br s, 2H, —CH_2), 2.23 (br s, 2H, —CH_2). IR (Nujol, cm^{-1}): 2924, 2854, 1651, 722. Anal. calcd. for $C_{24}H_{23}ClN_2O$ (in %): C-73.74, H-5.93, N-7.17. Found C-73.70, H-5.89, N-7.15%.

(4-benzhydryl-piperazin-1-yl)-(2,4-dichloro-phenyl)-methanone (9c). The general synthetic method described above afforded **9c**, and the product obtained was oily from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 2,4-dichlorobenzoyl chloride **7c** (0.41 g, 1.98 mmol). Yield(%): 65. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.68 (d, 1H, J = 1.85 Hz, Ar-H), 7.35–7.48 (m, 6H, Ar-H), 7.28 (t, 4H, J = 7.50 Hz, Ar-H), 7.18 (t, 2H, J = 7.24 Hz, Ar-H), 4.34 (s, 1H, —CH), 3.63 (br s, 2H, —CH_2—), 3.13 (br s, 2H, —CH_2—), 2.37 (br s, 2H, —CH_2—), 2.24 (br s, 2H, —CH_2—). IR (Nujol, cm^{-1}): 2929, 2862, 1670, 724. Anal. calcd. for $C_{24}H_{22}Cl_2N_2O$ (in %): C-67.77, H-5.21, N-6.59. Found C-67.73, H-5.17, N-6.55%.

(4-benzhydryl-piperazin-1-yl)-(3-bromo-phenyl)-methanone (9d). The general synthetic method described above afforded 9d, and the product obtained was off white crystalline solid from 1-benzhydryl-piperazine 5 (0.5 g, 1.98 mmol) and 3-bromo benzoyl chloride 7d (0.43 g, 1.98 mmol). Yield (%): 80, M.P (°C): 163–165.^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.62 (m 1H, Ar-H), 7.54 (s, 1H, Ar-H), 7.43 (d, 4H, J = 7.24 Hz, Ar-H), 7.35 (m, 2H, Ar-H), 7.28 (t, 4H, J = 7.60 Hz, Ar-H), 7.17 (t, 2H, J = 7.28 Hz, Ar-H), 4.32 (s, 1H, —CH), 3.64 (br s, 2H, —CH_2—), 3.34 (br s, 2H, —CH_2—), 2.31 (br s, 4H, —CH_2—). IR (KBr, cm^{-1}): 2935, 2842, 1670, 688. Anal. Calcd. for $C_{24}H_{23}BrN_2O$ (in %): C-66.21, H-5.32, N-6.43. Found C-66.17, H-5.28, N-6.39%.

(4-benzhydryl-piperazin-1-yl)-(2-fluoro-phenyl)-methanone (9e). The general synthetic method described above afforded **9e**, and the product obtained was yellowish oily from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 2-fluoro benzoyl chloride **7e** (0.30 g, 1.98 mmol). Yield (%): 80. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 8.0 (m, 3H, Ar-H), 7.82 (t, 1H, J = 1.17 Hz, Ar-H), 7.4 (d, 4H, J = 7.27 Hz, Ar-H), 7.25 (t, 4H, J = 7.35 Hz, Ar-H), 7.15 (t, 2H, J = 7.23 Hz, Ar-H), 4.3 (s, 1H, —CH), 3.67 (br s, 2H, —CH_2—), 3.24 (br s, 2H, —CH_2—), 2.34 (br s, 2H, —CH_2—), 2.23 (br s, 2H, —CH_2—). IR (Nujol, cm^{-1}): 3009, 2892, 1666, 1033. Anal. calcd. for $C_{24}H_{23}FN_2O$ (in %): C-76.98, H-6.19, N-7.48. Found C-76.94, H-6.15, N-7.44%.

(4-benzhydryl-piperazin-1-yl)-(2,6-difluoro-phenyl)-methanone (9f). The general synthetic method described above afforded **9f**, and the product obtained was oily from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 2,6-difluorobenzoyl chloride **7f** (0.34 g, 1.98 mmol). Yield (%): 77. ^{1}H NMR (DMSO-d6, 400 MHz) δ: 7.51 (m, 1H, Ar-H), 7.43 (d, 4H, J = 7.4 Hz, Ar-H), 7.28 (t, 4H, J = 7.56 Hz, Ar-H), 7.17 (t, 4H, J = 6.4 Hz, Ar-H), 4.35 (s, 1H, —CH), 3.68 (br s, 2H, —CH_2—), 3.24 (br s, 2H, —CH_2—), 2.36 (br s, 2H, —CH_2—), 2.23 (br s, 2H, —CH_2—). IR (Nujol, cm^{-1}): 2923, 2852, 1669, 1167. Anal. calcd. for $C_{24}H_{22}F_2N_2O$ (in %): C-73.45, H-5.65, N-7.14. Found C-73.40, H-5.61, N-7.09%.

(4-benzhydryl-piperazin-1-yl)-(3-methoxy-phenyl)-methanone (9 g). The general synthetic method described above afforded **9 g**, and the product obtained was off white crystalline solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 3-methoxybenzoyl chloride **7 g** (0.33 g, 1.98 mmol). Yield (%): 68, M.P(°C):120–122. ^{1}H NMR(DMSO-d6, 400 MHz) δ: 7.6 (m, 1H, Ar-H), 7.5 (s, 1H, Ar-H), 7.35 (t, 2H, J = 7.30 Hz, Ar-H), 7.3 (t, 4H, J = 7.41 Hz, Ar-H), 7.25 (t, 4H, J = 7.35 Hz, Ar-H), 7.15 (t, 2H, J = 7.26 Hz, Ar-H), 4.3 (s, 1H, —CH), 3.8 (s, 3H, —OCH_3—), 3.67 (br s, 2H, —CH_2—), 3.24 (br s, 2H, —CH_2—), 2.34 (br s, 2H, —CH_2—), 2.23 (br s, 2H, —CH_2—). IR (KBr, cm^{-1}): 3029, 2891, 1670, 1237, 1112. Anal. calcd. for $C_{25}H_{26}N_2O_2$ (in %): C-77.69, H-6.78, N-7.25. Found C-77.65, H-6.74, N-7.21%.

Table I. Inhibition zone (diameter mm) of synthesized compounds against tested bacterial strains by paper disc diffusion method.

\	Gram-positive bacteria				Gram-negative bacteria			
Compound	*B.cereus 11778*	*B.subtilis 6051*	*S. aureus* ATCC 25953	*S. epidermids* ATCC 25212	*P.aeruginosa ATCC 2853*	*E.coli ATCC 25922*	*P. vulgaris ATCC 2853*	*S. typhi ATCC 9484*
8a	13	16	14	15	12	12	14	15
8b	14	13	12	10	15	16	12	13
8c	12	13	15	11	11	12	11	13
8d	**27**	**28**	**26**	**27**	**22**	**21**	**22**	**25**
8e	**22**	**21**	**22**	**23**	**17**	**18**	**19**	**21**
8f	12	13	14	11	16	17	12	14
9a	11	13	12	14	20	17	21	19
9b	23	20	21	22	22	21	23	20
9c	**25**	**26**	**27**	**28**	**26**	**27**	**26**	**28**
9d	20	21	20	19	19	16	20	17
9e	**27**	**28**	**27**	**23**	**30**	**31**	**29**	**30**
9f	**29**	**27**	**26**	**30**	**29**	**26**	**28**	**28**
9 g	20	19	20	18	23	21	24	20
9 h	**26**	**25**	**28**	**29**	**27**	**25**	**26**	**26**
Streptomycin	**24**	**21**	**22**	**23**	**22**	**20**	**23**	**22**

(4-benzhydryl-piperazin-1-yl)-(3,5-dinitro-phenyl)-methanone (9 h). The general synthetic method described above afforded **9 h**, and the product obtained was white amorphous solid from 1-benzhydryl-piperazine **5** (0.5 g, 1.98 mmol) and 3,5-dinitro benzoyl chloride **7 h** (0.45 g, 1.98 mmol). Yield (%): 64, M.P(°C): 130–132. ^{1}H NMR(DMSO-d6, 400 MHz) δ: 8.82 (bs, 1H, Ar-H), 8.57 (d, 2H, $\mathcal{J}$ = 2.04 Hz, Ar-H), 7.43 (d, 4H, $\mathcal{J}$ = 7.24 Hz, Ar-H), 7.28 (t, 4H, $\mathcal{J}$ = 7.68 Hz, Ar-H), 7.18 (t, 2H, $\mathcal{J}$ = 7.28 Hz, Ar-H), 4.32 (s, 1H, —CH), 3.64 (br s, 2H, —CH_2—), 3.24 (br s, 2H, —CH_2—), 2.43 (br s, 2H, —CH_2—), 2.25 (br s, 2H, —CH_2—). IR (KBr, cm^{-1}): 3089, 2809, 1641, 1545, 1338. Anal. calcd. for $C_{24}H_{22}N_4O_5$ (in %): C-64.57, H-4.97, N-12.55. Found C-64.55, H-4.93, N-12.51.

Microbiology: In vitro *evaluation of antimicrobial activity*

The standard strains were procured from the American Type Culture Collection (ATCC) Rockville, USA, and the pathological strains were procured from the Department of Microbiology, University of Mysore, Mysore, India. The antibacterial activity of the synthesized compounds was screened against the following standard Bacterial strains: *Staphylococcus aureus* ATCC 25953, *Staphylococcus epidermis* 25212, *Bacillus cereus* 11778, *Bacillus substilis* 6021, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853, *Proteus vulgaris* ATCC 2853 and *Salmonella typhi* ATCC 9484.

Paper disc diffusion method. Preliminary antibacterial screening was performed by the agar diffusion method using a paper disc. The sterilized (autoclaved at 120°C for 30 min), liquified Mueller Hinton agar (40–50°C) was inoculated (1mL/100mL of medium) with the suspension of the microorganism (matched to McFarland Barium sulfate standard) and poured in to a Petri dish to give a depth of 3–4 mm. The paper discs impregnated with the test compounds (500 μg mL^{-1} in dimethylsulfoxide) were placed on the solidified medium. The plates were refrigerated at 4°C for 2 h and then incubated at 37°C for 24 h. The observed zones of inhibition (diameter) in mm are presented in **Table I**.

Minimum inhibitory concentration. A series of glass tubes containing different concentrations of the synthesized compounds (1–500 μg mL^{-1} in dimethylsulfoxide) with Mueller Hinton broth was inoculated with the required amount of the inoculum to obtain a suspension of microorganism, which contains 10^5 colony-forming units per milliliter. One growth control tube was prepared with the addition of the compound and one blank tube was prepared without the addition of microorganism. The tubes were incubated at 37°C for 24 h. The turbidity produced in each tube was recorded by using a UV- visible spectrometer. The minimum inhibitory concentration (MIC- μg mL^{-1}) was considered to be the lowest concentration, which exhibited the same turbidity as the blank tube. The observed MICs (μg mL^{-1}) are presented in **Table II**.

Results and discussion

Antimicrobial activity

1-Benzhydryl-piperazine derivatives **8(a–f)** and **9(a–h)** were synthesized and screened for their efficacy as antimicrobials against various pathogens

Table II. Minimum inhibitory concentration (MIC) in μg/mL of synthesized compounds against tested bacterial strains by micro dilution method.

	Gram-positive bacteria				Gram-negative bacteria			
Compound	*B.cereus 11778*	*B.subtilis 6051*	*S. aureus* ATCC 25953	*S. epidermids* ATCC 25212	*P.aeruginosa ATCC 2853*	*E.coli ATCC 25922*	*P. vulgaris ATCC 2853*	*S. typhi ATCC 9484*
8a	120	125	123	130	132	137	135	136
8b	132	130	135	131	144	110	135	140
8c	103	105	108	110	140	138	128	132
8d	80	85	86	79	72	80	82	73
8e	93	98	100	105	66	67	68	70
8f	144	120	123	150	85	82	73	74
9a	123	120	132	133	77	75	76	78
9b	95	96	98	99	82	85	83	88
9c	84	80	85	90	45	60	53	28
9d	64	65	68	70	96	98	80	17
9e	59	63	65	68	106	107	109	130
9f	43	48	49	60	79	78	79	28
9 g	60	63	68	65	142	165	130	128
9 h	50	55	60	53	131	163	114	131
Streptomycin	**157**	**210**	**234**	**134**	**154**	**178**	**147**	**195**

in vitro by paper disc diffusion and micro dilution methods. Streptomycin was used as a standard against both Gram-positive and Gram-negative bacteria. The results are shown in Tables I and II.

Piperazine analogues are known to have antibacterial activity [20,21]. Aromatic groups such as diphenyl methyl group attached to piperazine help in improving antibacterial activity of piperazine class of molecules by increasing lipophilicity as supported by our previous study [22]. At the same time, heterocyclic sulfonamides and carboxamides are reported to show antibacterial activity. Therefore in the present work 1-benzhydryl-piperazine sulfonamides/carboxamides derivatives are synthesised and screened for antibacterial activity.

In general, the synthesized compounds showed significant, moderate and less active inhibitory activity against pathogenic bacterial strains. Among sulfonamides **8(a–f)**, compounds **8d** and **8e** showed significant inhibitory activity against Gram-positive (zone of inhibition 26–28 mm, 21–23 mm respectively) and Gram-negative (zone of inhibition 21–25 mm, 17–20 mm respectively) bacteria. Compounds **8a** and **8f** showed moderate activity with the zone of inhibition in the range of 12–16 mm, 11–17 mm respectively. Compounds **8b** and **8c** shows less activity with the zone of inhibition in the range of 10–16 mm, 11–15 mm respectively. From the results obtained, it reveals that the presence of electron withdrawing groups (chloro in **8d** and **8e**) in the sulfonyl phenyl ring might be the reason for the significant inhibitory activity. In the presence of electron donating groups (tertiary butyl in **8f**) significant inhibition was not found. Compounds **8d** and **8e** showed observable antibacterial activity at the lower concentration of **72** μg/mL and **66** μg/mL against Gram-negative bacteria *P. aeruginosa* ATCC 2853.

Generally in benzamide series **9(a–h)**, compounds **9c, 9e, 9f** and **9 h** showed significant inhibitory activity in the order **9h** > **9c** > **9e** > **9f** against Gram-positive (zone of inhibition 26–30 mm, 25–29 mm, 25–28 mm and 23–28 mm respectively) and Gram-negative (zone of inhibition 29–31 mm, 20–29 mm, 26–28 mm and 26–27 mm respectively) bacteria. Compounds **9b, 9d, 9 g** showed moderate activity with the zone of inhibition in the range of 22–24 mm, 22–25 mm, and 24–29 mm respectively, where as the compound **9a** shows the less inhibitory activity.

From the results obtained, structure activity relationship can be drawn for sulfonamide series **8(a–f).** In this connection different electron donating or withdrawing groups attached to phenyl ring as substituents linked to sulfonyl group are studied for antibacterial efficacy. Upon introduction of the nitro group at the positions-2,-3 and -4 of the phenyl ring, the 2-NO_2 produces an enhanced activity probably by an ortho effect compared to the meta and para positions, indicating the positional requirement of nitro group on phenyl ring for enhanced activity in case of sulfonamide series **8(a–f).** Whereas the compound **8d** exhibited better activity against both gram positive and gram negative bacteria which has two electron withdrawing chloro groups at position-2 and -5 compared to **8e** which has only one chloro group at the 4-position. Compound **8f** showed moderate activity against both gram positive and gram negative bacteria might be due to the presence of electron releasing tertiary butyl group.

In carboxamide series **9(a–h)**, compound **9 h** having a nitro groups in positions-3 and -5 produces

good activity than other compounds in the series **9(a-h)**. Introduction of chloro groups in **9e** exhibits better activity against both gram positive and gram negative bacteria and fluoro groups in **9f** produces better activity against gram negative than gram positive bacteria. Similarly in the presence of bromo in **9d** and methoxy group in **9 g** showed moderate activity where as in the presence of tertiary butyl group in **9a** produces minimal activity.

As the electron negativity increases, the inhibition also increases as we observed from the derivatives **9c, 9e, 9f** and **9 h.** Significant inhibition was not found in the presence of electron donating groups in the amide ring. Compound **9 h** is most potent among the benzamide series **9(a–h),** showed observable antibacterial activity against *B. cereus* 11778 at 43 μg/mL, *B. substilis* 6021 at 48 μg/mL, *S. aureus* ATCC 25953 at 49 μg/mL and *S. epidermis* 25212 at 60 μg/mL. The structures of the potent antibacterials are summarised in **Figure 1**.

The structural correlation of the synthesized compounds reveals that, keeping the same substituents at the same position on the phenyl ring of both the sulfonamide and benzamide series **(8e, 9b)** and **(8f, 9a)** the sulfonamides show relatively significant antibacterial activity. Another structural correlation of the synthesized compounds is that, changing the substituents on the phenyl ring in the same position **(8c, 9b)** and **(8b, 9 g),** the carboxamides showed relatively significant antibacterial activity. This emphasises that, the nature of the functional linkage (-SO_2 - or –CO-NH-) influences the antibacterial activity. Finally, the above two structural correlation studies reveal that both nucleus and substituents are responsible for the antibacterial activity.

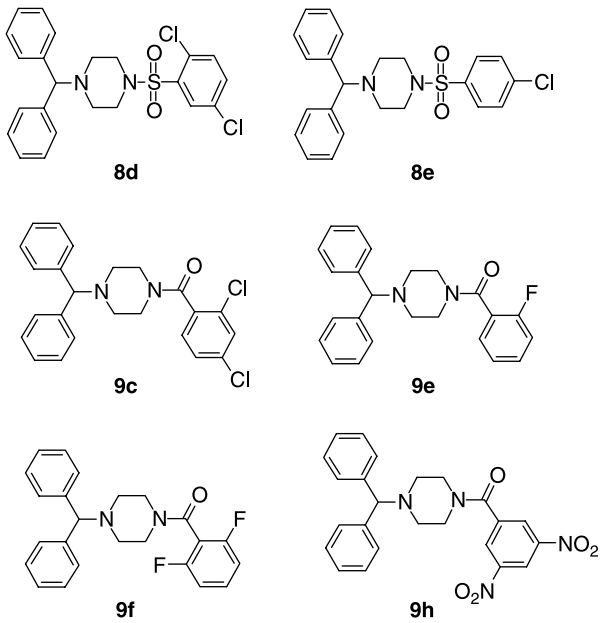


Figure 1. The structures of the potent antibacterials.

Conclusion

In conclusion, a series of novel 1-benzhydryl-piperazine derivatives **8(a–f)** and **9(a–h)** were synthesized in good yield and their antimicrobial activities were evaluated. Compounds **8d, 8e, 9c, 9e, 9f** and **9 h** demonstrated potent inhibition against all the strains tested. The two structural correlation studies reveal that, both linkage and substituents on phenyl ring are responsible for the antibacterial activity of these class of agents. Further synthesis of novel 1-benzhydryl-piperazine derivatives by changing the functional group on the basic scaffold to improve the antimicrobial activity is in progress in our laboratory.

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